used conforms to the standards prescribed by § 444.80(a)(1)(i), (iii), (iv), (v), and (vi) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing approximately 5.0 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dilute an accurately measured representative portion of the sample with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution containing 1,280 micrograms of tobramycin per milliliter.

### § 460.152 Trimethoprim concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Trimethoprim concentrated stock solutions for use in antimicrobial susceptibility test panels are frozen aqueous acidified trimethoprim lactate stock solutions serially diluted with distilled water to contain approximately the following concentrations: 6,400, 3,200, 1,600, 800, 400, 200, and 100 micrograms of trimethoprim per milliliter, or to a single concentration of 400 micrograms of trimethoprim per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of trimethoprim that it is represented to contain. The pH of the solution, containing 400 micrograms of trimethoprim per milliliter, is not less than 2.5 and not more than 6.0. The trimethoprim lactate used is a white, odorless, crystalline powder. Its potency is not less than 74 percent nor more than 78 percent trimethoprim. Its melting range is between 183° C and 187° C. Its loss on drying is not more than 1.0 percent. It passes the identity test. It conforms to the standards prescribed by this section.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The batch for potency and pH.

(*b*) The trimethoprim lactate used in preparing the solutions for potency, melting range, loss on drying, and identity.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 5 milliliters.

(b) *Tests and methods of assay*—(1) *Trimethoprim stock solution.* The sample solutions must be thawed and brought to room temperature before testing.

(i) *Potency*—(*a*) *Working standard.* Accurately weigh approximately 52 milligrams of the trimethoprim working standard. Dissolve and dilute the working standard in 100 milliliters of 0.1*N* hydrochloric acid to make a stock solution, containing approximately 400 micrograms of trimethoprim per milliliter. Further dilute the working standard twentyfold in distilled water to approximately 20 micrograms of trimethoprim per milliliter.

(*b*) *Preparation of sample.* The sample solution must be thawed and brought to room temperature. Further dilute with distilled water to an estimated concentration of 20 micrograms of trimethoprim per milliliter.

(*c*) *Procedure.* Using a suitable spectrophotometer equipped with 1.0 centimeter cells, determine the absorbance of the sample and working standard solutions at a wavelength of 270 nanometers.

(*d*) *Calculations.* Calculate the potency of the trimethoprim solutions as follows:

Micrograms of trimethoprim per milliliter in trimethoprim lactate=sample absorbance

times weight of standard (mg) times 0.763 times *f* times 1,000 times purity of standard in percent, divided by standard absorbance times 100 times 20 times 100

where:

*f*=dilution factor of each sample solution.

(ii) *pH.* [Reserved]

(2) *Trimethoprim*—(i) *Potency.* Proceed as directed in § 436.213 of this chapter using the method described in paragraph (e)(2) of that section except, to prepare the sample for assay, dissolve approximately 50 milligrams of sample accurately weighed in 60 milliliters of glacial acetic acid. Calculate the trimethoprim content as follows:

Percent trimethoprim=$(V_1-V_2)$ $(N)$ (290.3) (100) (100) divided by $(100-M)$ times $W$

where:

$V_1$=Volume perchloric acid used to titrate sample;
$V_2$=Volume perchloric acid used to titrate blank;
$W$=Sample weight in milligrams;
$N$=Normality of perchloric acid reagent;
$M$=Percent moisture in the sample.

(ii) *Melting range.* Proceed as directed in § 436.209 of this chapter.

(iii) *Loss on drying.* Proceed as directed in § 436.200(e) of this chapter.

(iv) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.25 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

[43 FR 9793, Mar. 10, 1978; 43 FR 12859, Mar. 28, 1978]

### § 460.153 Sulfamethoxazole concentrated stock solutions for use in antimicrobial susceptibility test panels.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sulfamethoxazole concentrated stock solutions for use in antimicrobial susceptibility test panels are frozen aqueous alkaline sulfamethoxazole stock solutions serially diluted with distilled water containing approximately the following concentrations: 12,160, 6,080, 3,040, 1,520, 760, 380, and 190 micrograms of sulfamethoxazole per milliliter, or to a single concentration of 760 micrograms of sulfamethoxazole per milliliter. The potency of each diluted solution is satisfactory if it is not less than 90 percent and not more than 140 percent of the number of micrograms of sulfamethoxazole that it is represented to contain. The pH of the solution containing 760 micrograms of sulfamethoxazole per milliliter is not less than 9.0 and not more than 12.5. The sulfamethoxazole used conforms to the standards prescribed by the National Formulary.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency and pH.

(ii) Samples required: A minimum of five frozen aliquots of each dilution of the concentrated stock solutions, each containing at least 10 milliliters.

(b) *Tests and methods of assay.* The sample solutions must be thawed and brought to room temperature before testing.

(1) *Potency.* Dilute aliquots of each sample in sufficient distilled water to make solutions containing 10 micrograms of sulfamethoxazole per milliliter. Place approximately 100 milligrams of the standard, accurately weighed, into a 100-milliliter volumetric flask and make to volume with 0.1*N* sodium hydroxide. Pipet 1.0 milliliter of this solution into a 100-milliliter volumetric flask and make to volume with distilled water. Using a suitable spectrophotometer equipped with 1.0 centimeter cells, and distilled water as the blank, determine the absorbance of sample and standard solutions at 257 nanometers. Calculate the potency of the sulfamethoxazole as follows:

Micrograms of sulfamethoxazole per milliliter=sample absorbance times weight of standard (in mcg) times *f* times purity of standard in percent divided by standard absorbance times 100 times 100

where:

*f*=Dilution factor of each sample solution.